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Larval Form and Metamorphosis of a "Primitive" Sea Urchin, *Eucidaris thouarsi* (Echinodermata: Echinoidea: Cidaroida), with Implications for Developmental and Phylogenetic Studies

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Abstract. The order Cidaroida (Echinodermata, Echinoidea) is universally recognized as an ancient (~230 mya) lineage and is thought to be the sister group to the more modern euechinoids. The present study on *Eucidaris thouarsi* corroborates earlier findings that cidaroids have a characteristic larval form that is different from that of euechinoids and gives the first detailed description of juvenile rudiment formation and metamorphosis in a cidaroid. Larvae of *E. thouarsi* lack an amniotic invagination (vestibule), have many (~20) juvenile spines on the larval epidermis and do not histolyze the entire larval epidermis at metamorphosis. Consequently, metamorphosis of cidaroid larvae is simple when compared to that of euechinoids. In larvae of *E. thouarsi*, epithelial cells appear to grow over the epidermis that becomes radial nerve tissue, but this process is not visible externally and may occur by a different mechanism than that reported for euechinoids. Typical development and metamorphosis of the class Echinoidea is usually represented by the euechinoids of the family Echinidae. The present study shows that feeding larvae of echinoids have greater variability than previously recognized in developmental patterns and processes, including differences in the fates of larval epidermal tissues and the timing of production of adult spines. The growth of podia exposed on the left side of the larval body is strikingly similar between cidaroid and asteroid larvae and is an example of probable convergence of characters among the echinoderms. The absence of a vestibule in cidaroids also raises uncertainties about the homology of this structure across the phylum Echinodermata.

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Introduction

Living echinoids form two distinct lineages, the Cidaroida and the Euechinoidea (Jensen, 1981; Smith, 1984a). Members of the extant family Cidaridae have a fossil record extending back into the Triassic (Karnian, *ca.* 230 million years ago). The cidarids are thought to have evolved from the miocidarids which had flexible tests with biserially arranged plates in each ambulacrum and interambulacrum, and interambulacral lantern supports called apophyses (Durham, 1966; Kier, 1977a; Jensen, 1981). Because miocidarids are the only echinoids known to have crossed the Permo-Triassic boundary, miocidarids are the presumed ancestors of the Euechinoidea (Durham, 1966; Kier, 1977a; Smith, 1984a). Recently, Kier (1984) showed that many of the Triassic echinoids lacked apophyses and therefore were not cidarids or miocidarids. Because some of these non-cidaroid Triassic echinoids showed slight development of ambulacral lantern supports (auricles), Kier (1984) proposed that the cidaroids and euechinoids actually separated prior to the first occurrence of the miocidarids, known from the Permian of Europe and North America (Kier, 1965, 1974).

Even though cidaroids and euechinoids have long been separate lineages and extant cidaroids are diverse (>140 species, Kier, 1977b), only a few studies have examined cidaroid larval development (*e.g.*, Prouho, 1887; Mortensen, 1921, 1937, 1938; Tennent, 1914, 1922; Schroeder, 1981). Mortensen (1938) reared *Prionocidaris baculosa* through metamorphosis; he described the

larva and the juvenile, but gave no detailed descriptions of juvenile rudiment formation or metamorphosis. McPherson (1968) described the larval development of *Eucidaris tribuloides* and included photographs of a larva and a juvenile (Figs. 23 and 24); however, the specimens in these photos are probably not cidaroids (see Discussion). Mortensen (1927) also described post-larval development in several cidaroids by examining small juveniles collected from the field. Barker (1985) provided a short description of the development of brooded embryos of *Goniocidaris umbraculum*. In contrast, over 45 species of euechinoids have been reared through metamorphosis (see Emlet *et al.*, 1987; Chia and Burke, 1978), and these studies provide the basis for the classical descriptions of echinoid development and metamorphosis (MacBride, 1903, 1918; von Ubisch, 1913; Hyman, 1955; Okazaki, 1975).

Recently, Schroeder (1981) studied the development of *Eucidaris tribuloides* from fertilization to the two-armed larval stage. He identified several unique aspects of development, confirmed Tennent's (1914) earlier observations that this species lacks early (primary) mesenchyme cells in the blastocoel, and pointed to the potential value of cidaroid developmental studies for understanding echinoderm phylogeny. Here I present a description of the larval development, larval form, metamorphosis, and early juvenile growth of *Eucidaris thouarsi* (Valenciennes), the eastern Pacific congener of the Atlantic *E. tribuloides*. These two species of *Eucidaris* are believed to have arisen from a common ancestor since the formation of the isthmus of Panama (Mortensen, 1928; Lessios, 1981). This study documents additional aspects of development in cidaroids that differ from developmental processes in euechinoids. Developmental and phylogenetic implications for all echinoids and for echinoderms in general are discussed.

Materials and Methods

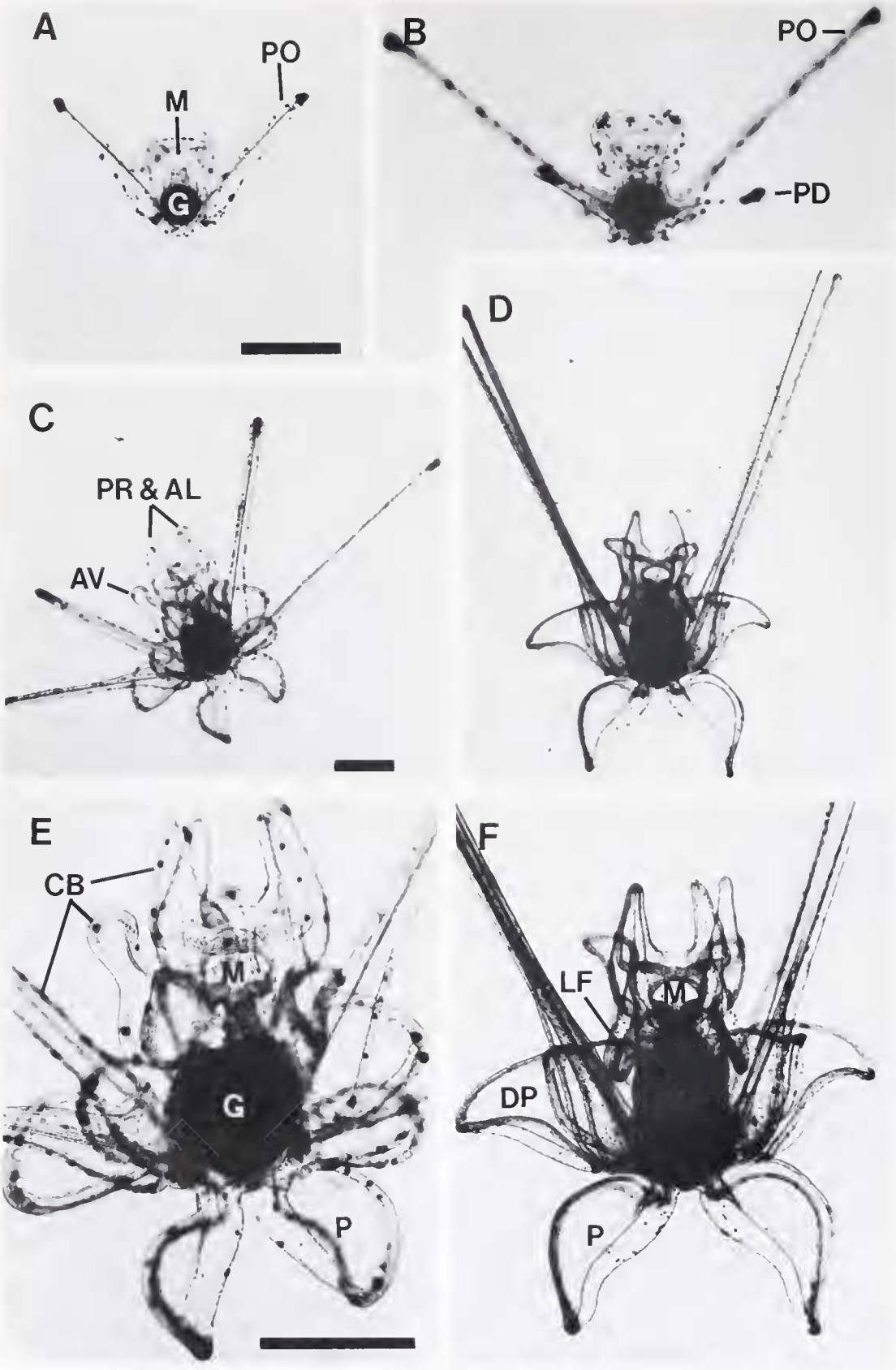
Adult specimens of *Eucidaris thouarsi* (Valenciennes) were collected at 3 to 5 m depth at Isla Taboguilla, Bay of Panama, Republic of Panama. Urchins were induced to spawn by intracoelomic injection of 5 to 10 mls of isotonic (0.55 *M*) KCl. Eggs were washed three times in filtered water (0.25 μm) and fertilized with several drops of a dilute sperm solution. Larvae were cultured in liter or gallon glass jars at a temperature of 28°C and salinity of 30‰ according to Strathmann's (1971) methods. Filtered water was changed on alternate days. Larvae were fed a combination of *Dunaliella tertiolecta* Butcher and *Rhodomonas lens* that were grown in a culture medium enriched with Alga-Gro Concentrate (Carolina Biological, Inc). Larvae were fed daily with algal cells that were first centrifuged and then resuspended in filtered seawater. Although no attempt was made to measure food concentrations, full larval guts indicated that adequate food was available. Terminology used for larval arms and spicules is from Mortensen (1921).

Observations of metamorphosis were made on late larvae with well-developed primary podia and juvenile spines. Larvae were put into small glass bowls with approximately 40 ml of filtered seawater. Each bowl contained a small piece of rock (1–2 cm^2) with organic (biological) films and encrusting algae (coralline and non-coralline red algae). Rocks were collected from adult habitats and held in the laboratory seawater table prior to use. Upon introduction to settlement bowls, larvae were observed for several minutes. They were then checked every 15 minutes for the next several hours to observe metamorphosis.

Larvae at different stages of development, including several that were metamorphosing, and several juveniles were fixed for 1 hour in buffered formalin (3%) and preserved in 70% EtOH. [Though other fixatives are superior to formalin, they were not available at the time the animals were preserved. It should be noted that the classic study by von Ubisch (1913) of the metamorphosis of the euechinoid *Paracentrotus lividus* was conducted on specimens fixed in buffered formalin. Furthermore, von Ubisch's results agree very well with studies on other euechinoids fixed in osmic acid and Müller's fluid (MacBride, 1903).]

Specimens from among these samples were prepared for observation on a scanning electron microscope (SEM) by critical point drying and coating with gold-palladium. Still other preserved specimens were prepared and serially sectioned according to the following methods. Specimens were decalcified in 5% EDTA (ethylenediaminetetraacetic acid) adjusted to pH 7.0 with NaOH, dehydrated through an alcohol series, and embedded in Spur embedding medium (Polysciences, Inc). Sections (3–5 μm thick) were cut in the frontal plane of the larvae, thus passing through the oral-aboral axis of the future juvenile. Sections were cut through juveniles in a similar orientation, the first sections passing through ambulacrum III. All sections were stained for 10 min with Harris' hematoxylin as modified by S. C. Chang (Smithsonian Institution), rinsed in running water, and counterstained for 10 min with basic fuchsin as modified by S. C. Chang. This staining protocol made it possible to identify nuclei, cytoplasm, some cytoplasmic granules, muscle cells, and collagenous connective layers.

The crystal orientations of larval spicules and apical plates were determined in late stage larvae and newly metamorphosed juveniles by observing them with polarized light on a microscope fitted with a Universal stage. The direction of the crystallographic axis was determined according to the methods of Emmons (1943). To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increase the transmission of light through soft tissues, specimens were first dehydrated and then put into unpolymerized embedding medium prior to observation under polarized light.

Results

Early development and larval form

The eggs obtained from 3 females had mean diameters of 87.7 μm (SD = 1.76, n = 25), 87.8 μm (SD = 4.70, n = 25), and 93.6 μm (n = 3). Early cleavage and development followed a pattern similar to that reported for *Eucidaris tribuloides* (Tennent, 1914; Schroeder, 1981). No mesenchyme cells were evident in the blastocoel until the gastrula stage when the archenteron extended halfway into the blastocoel. Spicules appeared by 28 h after fertilization. Larvae reached the two-armed stage and were feeding by 73 h after fertilization. Postoral arms grew laterally at first; as development continued, these arms became oriented more anteriorly but were still widely spread (Fig. 1A).

Table I summarizes observations on the development of two larval cultures of different parentage. The given times are observation times and do not necessarily represent the initiation of the reported events. In general, the variation in larval stages within cultures increased with time. At no time was there any indication that general culture conditions were poor or that culture conditions produced artifacts that might be misinterpreted in the results. Throughout the study when they were observed with dissecting and compound microscopes, larvae appeared to be healthy and progressing through developmental stages. After 30 days, when the first larvae metamorphosed, others had partially developed juvenile structures and still others showed no external sign of juvenile rudiment development.

The two-armed stage persisted through the 10th day after fertilization (Fig. 1A). During the development of the postoral spicules and arms, the calcareous rods associated with the anterolateral arms grew from the same spicules. These rods grew into the preoral hood region of the larva, but anterolateral arms that project anteriorly from the preoral hood region had not yet formed. Twelve to 15 days after fertilization, the posterodorsal arms formed and lengthened (Fig. 1B). By Day 15 some larvae had anterolateral arms, the dorsal arch spicule extended into the preoral hood region where "buds" of preoral arms were evident, and an unpaired posterior transverse spicule was present (Table I).

Morphology and behavior of late-stage plutei

From this point larval form began to depart markedly from the patterns typical of euechinoid larvae. Larvae of *Eucidaris thouarsi* developed epidermal lobes at specific locations on the body. Five pairs of lobes formed, all at locations along the ciliated band. Two pairs formed on each of the ventral and dorsal surfaces of the larval body; a fifth pair formed at the posterior end of the larval body, between the posterodorsal and postoral arms (Figs. 1C–F; 2A, C). Much of the rest of larval growth consisted of the lengthening of larval arms, including the anterolateral and preoral arms, and the enlargement of the epidermal lobes. A pair of lateral flaps extended from the preoral hood in a postero-ventral direction (Figs. 1D, 2A). Fully developed larvae had eight arms, five pairs of epidermal lobes, paired flaps on the preoral hood (Figs. 1D, 2A) and were approximately 2.5 to 3 mm long from posterior edge of the posterior lobes to the tip of postoral arms.

In larvae with developing lobes, the longer (postoral and posterodorsal) arms could be moved by muscles at the base of the spicules and posterior to the stomach (Figs. 3B, I). If the culture bowl was moved and the water disturbed, if the jar containing the larvae was tapped, or if larvae were subjected to suction from a pipette, larvae underwent a dramatic change in shape by moving the postoral and posterodorsal arms in unison from an anterior to a posterior orientation. Larvae flared these arms

Figure 1. Light micrographs of larval stages of *Eucidaris thouarsi*. All scale bars = 300 μm. A. Ten-day-old larva with a pair of postoral arms. B. Fifteen-day-old larva with two pairs of arms (same scale as A). C. Twenty-day-old larva with characteristic cidaroid lobes forming (ventral view). D. A ventral view of a twenty-nine-day-old larva with lobes highly developed but no juvenile structures (same scale as C). E. Higher magnification detail of C. F. Higher magnification detail of D (same scale as A).

Symbols used in Figures 1 through 4: *Larval arms and lobes:* AL, anterolateral arms; PD, posterodorsal arms; PO, postoral arms; PR, preoral arms; AD, antero-dorsal lobes; AV, antero-ventral lobes; DP, dorso-posterior lobes; VP, ventro-posterior lobes; P, posterior lobes; LF, lateral flap. *Other larval structures:* BL, blastocoel; CB, ciliated band; G, larval gut; H, left hydrocoel; LC, left posterior coelom; M, larval mouth; MU, skeletal muscles; Œ, esophagus; PT, posterior transverse rod; #, epidermis contracting along larval spicules. *Adult structures:* AS, adult spines; B, (podial) bud from water canal; C, perivisceral coelom; D, epidermis of oral disk; EL, epithelial layer; ES, epineural space; JS, juvenile spines; O, ocular plate; PB, primary podial bud; PC, epidermal pigment cells; PED, pedicellaria; RN, radial nerve; S, spine attached to genital plate 1; W, water ring; 1,2,3,4,5, genital plates 1–5.

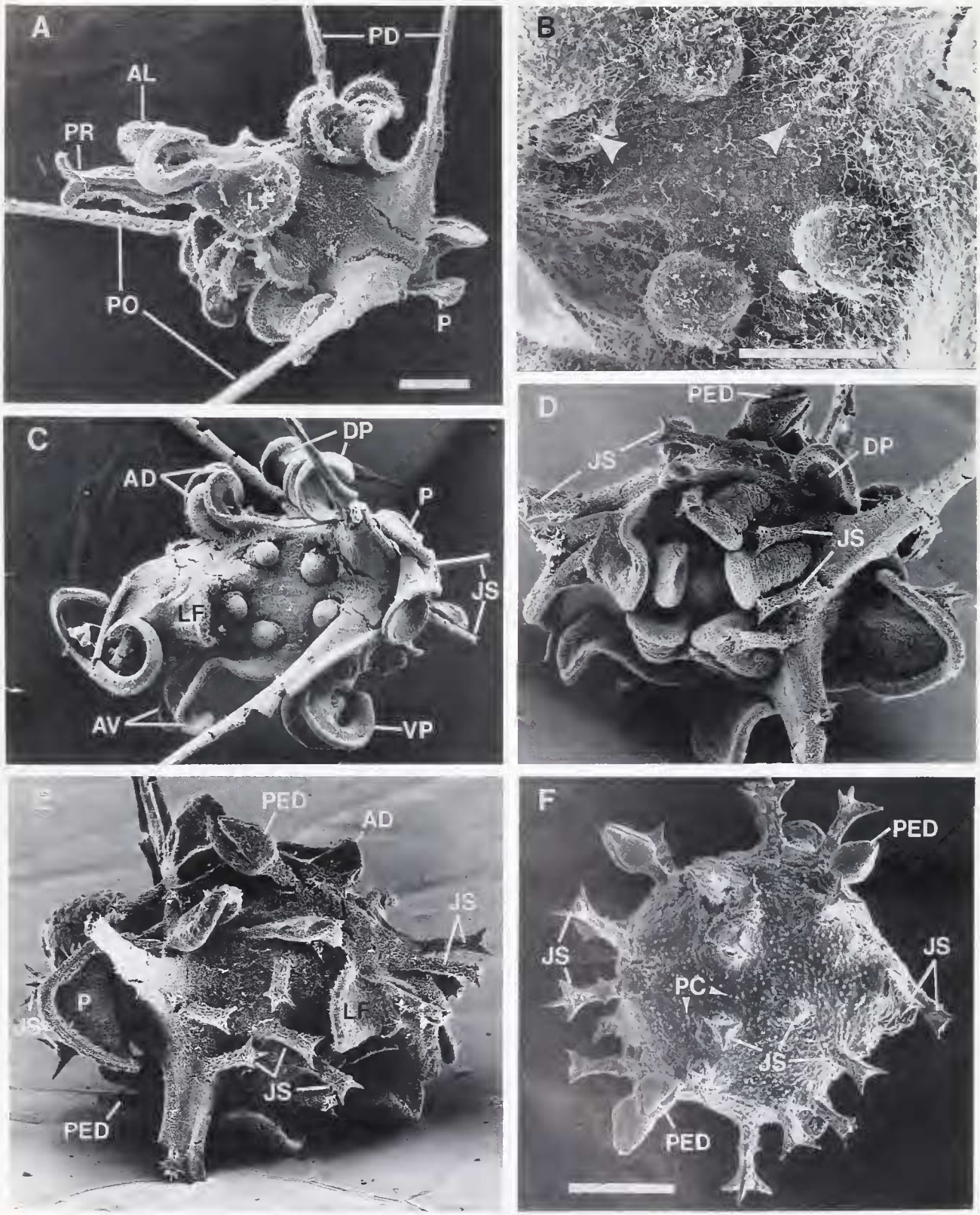

Figure 2. Scanning electron micrographs of larvae of *Eucidaris thouarsi* A–D show progressive development of the primary podia which, in the absence of an amniotic sac, are exposed on the left side of the

Table I

Summary table of developmental events of Eucidaris thouarsi (*Valenciennes*)

Time since fertilization	Developmental stage or event
1.75 h	Four and eight cell embryos.
19 h	Early to mid gastrula. Archenteron extends 1/3 to 1/2 way into blastocoel. Mesenchyme absent.
21.5 h	Gastrula. Archenteron extended halfway into blastocoel. Mesenchyme is present. No spicules.
28 h	Late gastrula. Archenteron has contacted the blastocoel wall. Spicule primordia evident.
65 h	Prism/early two-armed larvae. Gut formation complete. Postoral spicules 115 μm long.
73 h	Feeding two-armed larvae. Stage is similar to that reported by Schroeder (1981, Fig. 3 O, P, Q).
10 days	Two armed larvae. Postoral arms well developed, 470 μm long (measured from posterior curve in ciliated band to arm tip).
11 days	Initiation of paired posterodorsal and unpaired dorsal arch spicules.
15 days	Posterodorsal arms are less than half the length of postoral arms. Buds of preoral arms and posterior transverse spicule are present.
18 days	Epidermal lobes are beginning to form.
20 days	Lobes continue to develop. Anterolateral and preoral arms grow anteriorly from the preoral hood.
25 days	Epidermal lobes are very highly developed. Postoral and posterodorsal arms approx. 2 mm long. The most advanced larvae have five primary podia, four pedicellariae, and two posterior juvenile spines.
27 days	More larvae have primary podia and pedicellariae, including one on the dorsal surface; juvenile spines are present in several locations on the larval body. Most advanced stages show reductions in arm length and lobes.
30 days	First larvae metamorphose. Juvenile test diameter approx. 510 μm.

Cultured larvae grew at 28°C and salinity 30 ppt.

outward and then backward, moving each arm through approximately 90 degrees of rotation. This flaring movement was rapid and temporary, with arms moved from the original position to the posterior position and back to the anterior position in approximately 1 s. Often the flaring reaction was repeated several times (2 to 5 or more times). During the flaring movement the larval body was thrust anteriorly while the arms moved posteriorly; as the arms returned to an anterior position the larval body moved posteriorly. After single or multiple flarings, there was no net movement of the larva. Larvae were never observed to use this flaring movement as a means of locomotion. Throughout development in culture bowls, larvae swam with their anterior ends upward. Maintaining this orientation, they moved slowly up or down with the aid of currents produced by the ciliated band.

Development of juvenile structures

The first juvenile structures to form were pedicellariae and embryonal or juvenile spines (Table I). A pair of pedicellariae formed at the bases of the posterodorsal arms on the dorsal surface of the larva. Another pair of pedicellariae formed at the bases of the postoral arms on the ventral surface. At the extreme posterior end of the larva, between these two pairs of pedicellariae, a pair of juvenile spines formed in association with the posterior transverse rod (Fig. 2C). No pedicellariae formed on this last rod. Several days later, a single pedicellaria and two juvenile spines formed on the dorsal surface of the larva over the region of the dorsal arch spicule (Fig. 2D).

As these structures were forming, buds of primary podia emerged on the left surface of the larval body (Fig. 2B, C). These buds were not enclosed in a vestibule or amniotic sac, but were exposed on the larval surface. Observation with SEM of the left surface of two- and four-armed larvae (not pictured) failed to show an invagination or cellular irregularity on the left surface that might indicate an amniotic invagination. Podia appeared first as bulges on the left surface (Fig. 2B). Over approximately 5 days, the podial buds grew into functional tube feet with terminal discs (Figs. 2C, D). After this time po-

larval body. Note also the lack of external evidence for epineural folds. A. Eight-armed larva with well-developed lobes, but without juvenile structures. Anterolateral arms folded during preparation for SEM (scale bar = 100 μm). B. A higher magnification of left surface of body region of an older larva shows the first external evidence of podial buds. Arrows indicate two of five buds that are less well developed (scale bar = 50 μm). C. Larva with podial buds at an intermediate stage of development (same scale as A). D. An advanced larva with primary podia that have well-formed terminal discs (same scale as A). E, F show an advanced larva from right side and a juvenile in the corresponding orientation (with interambulacral [illegible] on the left). E. Right side view of same advanced larvae as D. The two posterior pairs of larval arms have been broken off to allow orientation of the larva (same scale as A). F. A juvenile, one day after metamorphosis; the only spines present are juvenile spines. The corresponding spines and pedicellariae are marked in 2E and F and show that only small positional changes occur at metamorphosis (scale bar = 100 μm). See legend to Figure 1 for symbol identification.

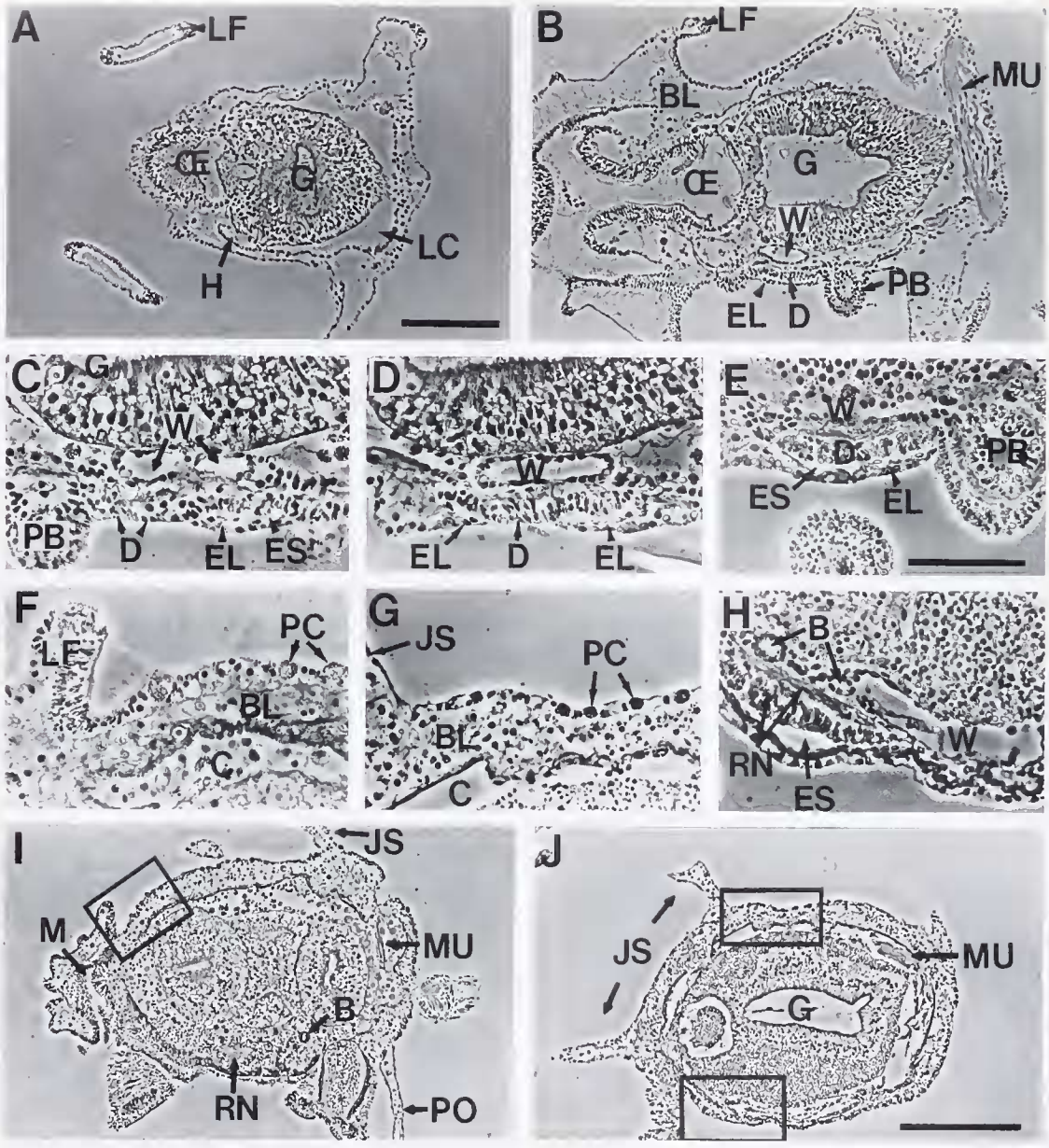

Figure 3. Histological sections of larvae and juveniles, phase contrast photomicrographs. All sections through larvae are frontal sections passing through the developing oral region of the juvenile. Each photograph is oriented with the larva's posterior (or corresponding part of the juvenile) on the right, and with the larva's left side (or juvenile's oral side) at the bottom. A. An early eight-armed larva with the hydrocoel as a simple sac on the left, anterior side of the gut (scale bar = 100 μm). B, C, D. An ordered sequence of sections through a larva with initial buds of primary podia and with epithelial layers partially covering the epidermis of the oral disk. B. A section, dorsal to the medial plane, passing tangentially through the water ring and through part of two podial buds. At the level of this section the epithelial layer covers the oral disc (same scale as A). C. Higher magnification of a section passing through a more medial region of the water ring (note 2 lumens are present and the labeled podial bud is ambulacrum II). The epidermis of the oral disk is exposed on the left but is covered by an epithelial layer on the right side (same scale as E). D. Section, ventral to the medial plane, passing tangentially through the water ring. At this location epithelial layers (and underlying epineural spaces) are on the left and right of the oral disk which is exposed in the middle of the photo, below the water ring (same scale as E). E. A later larval stage, with the oral disk completely enclosed by the epithelial layer (scale bar = 50 μm). F. A section through the epidermis of a competent larva. The approximate location of this section is indicated by the box in I (same scale as E). G. A section

dia were seen to move and eventually extend out from the larval surface by muscular contraction.

Histological sections through eight-armed larvae without podial buds showed the larval epidermis as a simple epithelium of cuboidal cells, and showed a pouch-shaped left hydrocoel located on the left side of the stomach (Fig. 3A). Before the podial buds began to form, the left hydrocoel developed into the ring canal, but the details of this process were not followed. By the time podial buds formed, the larval epidermis on the left side of the larval body was a stratified epithelium, several cell layers thick, and matched the description of the "ectodermic" or "oral disc" of euechinoids (Theel, 1902; MacBride, 1903). Together with hydrocoelic lobes that became the radial canals, the epidermis of the oral disc formed five evaginations that were the beginnings of the podial buds. Serial sections through larvae in early stages of podial bud formation showed a simple epithelium overlying the epidermis of the oral disc at its periphery and between the podial buds. At this stage, the center of the oral disc lacked this epithelial cover (Figs. 3B–D). The parts of this epithelium closest to the center of the oral disc were thin, had few nuclei, and appeared to be one cell thick. The epithelial cells tapered to an acute angle at their medial edges on the disc and here the epithelium lay flat on the epidermis of the oral disc (Figs. 3C, D). In this region the epithelium appeared to be continuous with the underlying epidermis of the oral disc, but could be distinguished from it by the orientations of the nuclei and by the staining properties of the cytoplasms. Peripheral to this thin layer, the epithelium was stratified into two layers, each one cell thick. In this location gaps between the epithelium and the epidermis of the disc opened, as did gaps between the two cell layers of the epithelium (Figs. 3C, D). The gaps between the epithelium and the epidermis of the oral disk correspond to the epineural space of euechinoids. In a slightly more advanced specimen (with longer podial buds) the epithelium completely covered the oral disc, however the podial buds remained free of this epithelium (Fig. 3E). In this specimen the epineural space between the epidermis and the epithelial layer was continuous but narrow across the center of the oral disc. A darkly stained connective tissue lay between the epithelial cell layers and there were few gaps between the epithelial layers (Fig. 3E).

At no time during the development of primary podia was there external evidence of the epithelium encroaching on the oral disc epidermis. SEM observations showed a complete, evenly ciliated and flat surface in and around the oral region (Fig. 2B). There was no indication of tissue folds forming lobes (with free edges) and moving into the region of oral disc, as has been reported in the formation of the epineural cavity of euechinoids (Theel, 1902; MacBride, 1903; von Ubisch, 1913; Hyman, 1955).

As the podial buds developed, the larval epidermis of the oral disc thinned in the center and thickened in areas around the ring canal and radial canals. Between the thickened regions of larval epidermis and the ring and radial canals of the hydrocoel, a region formed that appeared to contain fiberous material (Figs. 3E, H). The topographical arrangement of these tissues was similar to the description of the nerve ring and radial nerve given for the euechinoid *Paracentrotus lividus* by von Ubisch (1913).

In the most advanced larval stages, podia were well developed, and numerous juvenile spines could be seen on the dorsal, ventral, and right surfaces of the larvae (Fig. 2D, E). At least 19 juvenile spines were counted in the epidermis of the larva pictured in Figure 2E. In the larvae raised through metamorphosis, no definitive or adult spines were present on the larvae prior to metamorphosis (Figs. 2E, 3I). Advanced stage larvae also had shortened larval arms and greatly reduced epidermal lobes (Figs. 2D, E).

Histological sections through larvae that were judged capable of metamorphosis failed to show evidence of Aristole's lantern, adult spines, or their primordia. The lobes of the left posterior coelom extended interradially toward the oral disc, and were similar to the dental sacs reported in similar stages of regular euechinoids (Theel, 1902; MacBride, 1903; von Ubisch, 1913). The radial water canals showed small buds of additional podia subterminal to the well developed primary podia (Figs. 3H, I). The larval epidermis of the late larvae was consistently thicker than earlier stages and contained numerous large pigment-bearing cells (Figs. 3G, I).

Metamorphosis

Advanced larvae metamorphosed within 2 h after being introduced to a small bowl containing filtered seawater and rock substrata. Larvae at similar stages never

through juvenile aboral epidermis, three hours after metamorphosis. This section is an enlargement of the region indicated by the upper box in J (same scale as E). H. A section through the oral surface of juvenile three hours after metamorphosis. The approximate location of this section is indicated by the lower box in J (same scale as E). I. Section through a competent larva (same scale as J). J. Section through a newly metamorphosed juvenile. Comparison with I shows that only small rearrangements and resorption in place of larval structures is required to transform a competent larva into a juvenile (scale bar = 200 μm). See legend to Figure 1 for symbol identification.

metamorphosed in the larval culture bowls; they appeared to react to the presence of the rock substratum. Larvae that swam over the rock would often sink and make contact with the rock. Upon contacting the rock, larvae would flare their arms and hold them in the posterior position. At this point the larvae would attach their podia to the substrate. This posture always preceded metamorphosis, but occasionally larvae were also seen to release from the rock and to begin swimming about the culture dish with arms again directed anteriorly.

When metamorphosis began it was accompanied by a waving movement of the posteriorly directed arms, followed by a contraction of the larval epidermis down the postoral and posterodorsal spicules (Fig. 4A). When the epidermis had retracted most of the way down these spicules, these four rods were severed at their bases. The straight portions of the rods fell away from the epidermis, and their bases remained on the right (aboral) and posterior (lateral) surfaces of the larva (juvenile). The next external changes to occur were the loss of the larval form by resorption of the remaining ridges and lobes, contraction of the epidermis from other larval spicules, erection of the juvenile spines, and a rounding up of epidermis to attain the shape of a juvenile echinoid (compare Figs. 2E, F).

Despite changes in shape, much of the larval epidermis in the body region remained intact and became the juvenile epidermis. There is both indirect and direct evidence for this. Indirect evidence that the larval epidermis became the juvenile epidermis is based on the topologies of the late larvae and the early juveniles. Both these stages bear numerous juvenile spines and pedicellariae, lined with larval epidermis. These structures act as surface markers and show that very little rearrangement of the surface took place prior to and within 1 day after metamorphosis (Figs. 2E, F). Direct evidence that much of the larval epidermis remained after metamorphosis is based on a comparison of histological sections through competent larvae and through juveniles (3 h after metamorphosis). Again, these sections (Figs. 3I, J) indicate that resorption of larval structures in place is all that occurred to transform the larva into the juvenile. A continuity of the epidermis between these two stages was evident from the large pigment-bearing cells interspersed among thick, cuboidal epidermal cells (Figs. 3F, G). Sections show numerous cells within the blastocoel; many of these are probably the degenerating remains of resorbed larval arms and lobes.

The final skeletal change that occurred during the shape rearrangements of the epidermis was a return of the bases of the postoral and posterodorsal spicules to the orientations they had when the arms were directed anteriorly. This return of the bases of the postoral and posterodorsal rods to their pre-metamorphic positions was verified by observation of larvae and juveniles under polarized light. The crystallographic axes (C-axes) of the calcite in the postoral and posterodorsal rods coincide with the long axes of the rods (see also Emlet, 1985). The orientations of the C-axes of the spicule bases indicated their return to their original positions. Because of this movement of the spicule bases, all of the larval skeletal elements that remained in the juvenile were in positions they had occupied prior to metamorphosis.

Observations of the metamorphosing larvae and of the early juveniles (within 5 h of the initiation of metamorphosis) under polarized light showed little change in the positions of the bases of larval spicules. (Fig. 4A–C). During the next 48 hours, skeletal (genital) plates covered the aboral surface by medial growth, not by migration, of the skeletal elements (Fig. 4C–F). In addition, adult spines formed and grew from interambulacral plates that formed after metamorphosis (Fig. 4E, F).

Discussion

Comparison of development among cidaroids

This study on the development of *Eucidaris thouarsi* supports Mortensen's (1937) conclusion that cidaroids have a larval form distinct from that of other echinoids. Larvae of *Cidaris cidaris* (Prouho, 1887), *Eucidaris metularia* (Mortensen, 1937), and *Prionocidaris baculosa* (Mortensen, 1938) have all been described and figured with characteristic lobes associated with the ciliated band. It is on this basis that Mortensen (1937) suggested that the Mediterranean larva pictured by Müller (1854) was that of a cidaroid, either *Stylocidaris affinis* or *Cidaris cidaris*. In addition, Mortensen (1937) stated that a larva he originally identified as *Astropyga pulvinata* (Mortensen, 1921, Pl. V, Fig. 7) was also a cidaroid, probably *Eucidaris thouarsi* (due to the locality—Bay of Panama). Though some euechinoid larvae do develop ciliated lobes (see below), only in cidaroid larvae are the lobes so numerous and well developed. The lobes give cidaroid larvae a more elaborate shape than most euechinoid larvae. The high degree of development of these fleshy lobes and the elaborate ciliated band are reminiscent of some asteroid (bipinnaria) larvae. An arm flaring behavior similar to that of *E. thouarsi* was described and figured for larvae of *Prionocidaris baculosa* by Mortensen (1938, Plate II, Fig. 2).

All cidaroids probably lack an amniotic invagination (or vestibule) on the left side of the larval body. Descriptions of the development of juvenile structures in feeding larvae of *Eucidaris metularia* (Mortensen, 1937) and brooded embryos of *Goniocidaris umbraculum* (Barker, 1985) stated that podia could be seen. Neither study mentioned presence or absence of an amniotic sac. The lack of a vestibule in *E. thouarsi* is correlated with the

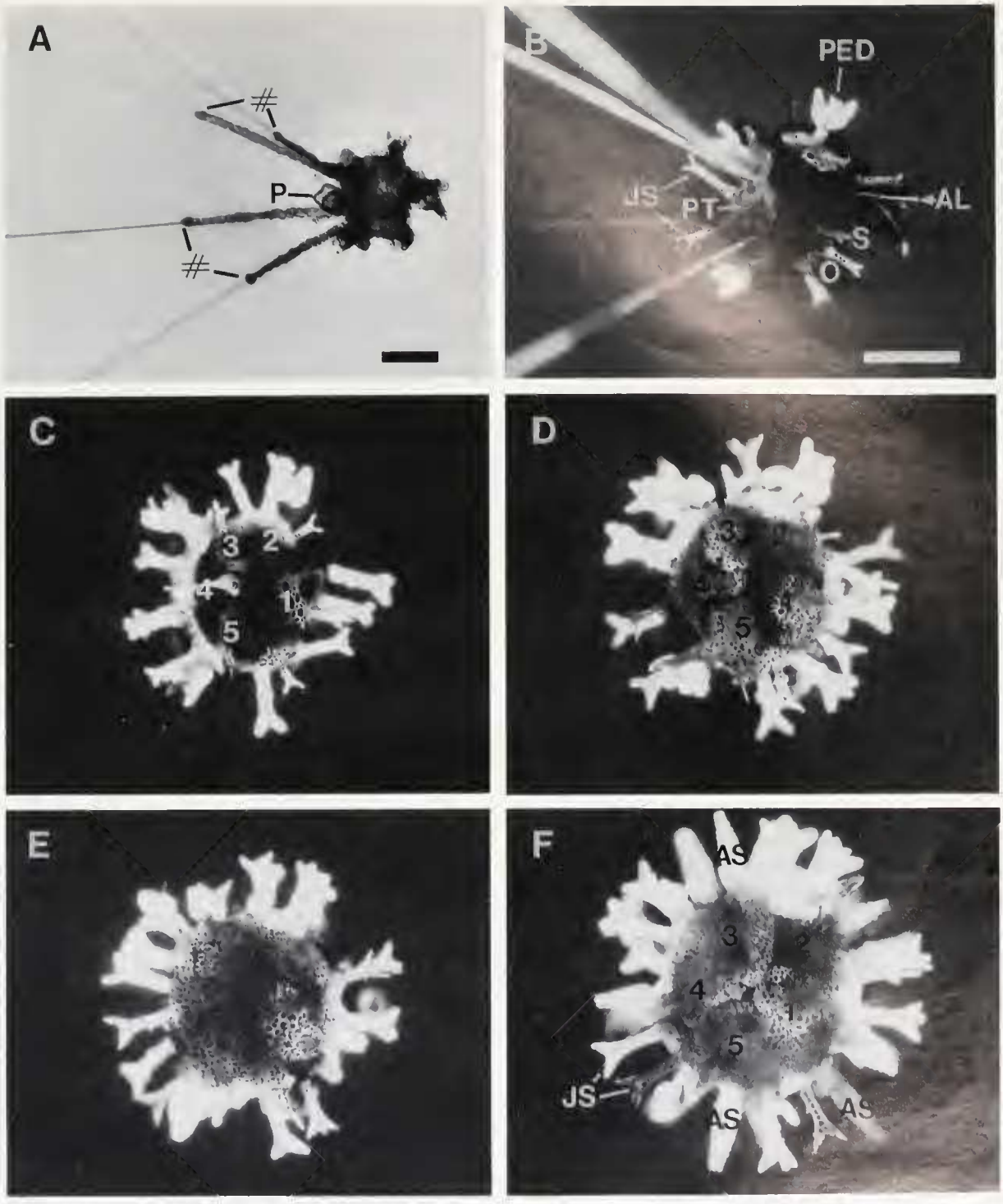

Figure 4. Light micrographs of a metamorphosing larva (A, B) and newly metamorphosed juveniles of *Eucidaris thouarsi* (C, D, E, F). All photographs are oriented with the posterior end of the larva and the corresponding part of the juvenile on the left side of the picture. Scale bars = 300 μm, B–F are all the same magnification. A, B. A larva during metamorphosis (right lateral view). B. Calcified larval and juvenile structures are revealed by polarized light. At the posterior end of the larva, two juvenile spines are attached to the posterior transverse rod. The bases of right postoral and posterodorsal rods are positioned at the periphery of the juvenile aboral surface to either side of a part of the posterior transverse rod. C. Juvenile 5 hours after metamorphosis. Numbers 1–5 indicate genital plates; four of these form from larval skeletal elements. D, E. Juveniles approximately one day after metamorphosis. The aboral surface of these juveniles is gradually being filled by the growth of apical plates. E is slightly more advanced than D. F. Juvenile, two days after metamorphosis. The apical system has almost completely filled the aboral surface. Adult spines can be seen at the circumference of the test and have a very different morphology from juvenile spines. See legend to Figure 1 for symbol identification.

numerous juvenile spines and absence of adult spines at metamorphosis. A similar morphology is seen in late larvae and early juveniles of *Prionocidaris baculosa* (Mortensen, 1938). Two cidaroids with large yolky eggs, *Phyllacanthus parvispinus* and *P. imperialis*, also lack signs of an amniotic invagination during modified development through a non-feeding larval stage (R. Raff, pers. comm.; R. Olson, pers. comm.). Because the euechinoid *Heliocidaris erythrogramma*, with non-feeding larval development has an amniotic invagination (Williams and Anderson, 1975), the absence of a vestibule in species of *Phyllacanthus* is probably a cidaroid trait and not due to modified development. The uniformity of morphology among feeding larvae of cidaroids supports my contention that the absence of a vestibule is typical of cidaroids.

McPherson's (1968) study of the biology of *Eucidaris tribuloides* included photos of a 20-day-old larva (Fig. 23) and a juvenile 15 days after metamorphosis (Fig. 24). It is unlikely that either of the photographed specimens are *E. tribuloides*. The larva pictured by McPherson lacks the highly developed lobes found on other cidaroid larvae including *Eucidaris metularia* and *E. thouarsi* (Figs. 1D, 3). Even McPherson acknowledged that "lobes were not as conspicuous as those described for *E. metularia* by Mortensen (1937)" (pg 430). The larva pictured by McPherson has a well-developed pedicellaria on the posterior transverse rod, but lacks the juvenile spines at the posterior end. Larvae of *E. thouarsi*, *E. metularia*, and *P. baculosa* always have a pair of spines at the posterior end. These larvae also have one pedicellaria at each of the bases of the posterodorsal and postoral body rods, but not on the posterior transverse rod. The similarity between cidaroid larvae in general, and especially between larvae of *Eucidaris metularia* and *E. thouarsi*, makes it unlikely that the larvae of *E. tribuloides* would differ in the ways reported by McPherson (1968). One larva in McPherson's culture metamorphosed and lived for 15 days. He noted that the juvenile had two types of spines and ambulacral structures resembling sphaeridia, and he remarked on their apparent absence in other cidaroids. The juveniles of *E. thouarsi* reared in the present study never grew structures resembling sphaeridia, and though juveniles had two types of spines, the adult spines differed in morphology from those of the juvenile pictured by McPherson (1968). The larva and juvenile pictured by McPherson (1968) appear to be those of a euechinoid, possibly *Echinometra* sp. or *Tripneustes* sp.

Comparisons of cidaroid and euechinoid larvae

Cidaroids and euechinoids differ in a number of ways throughout development (Table II). Raff *et al.* (1984) showed that euechinoids possess maternal α-subtype histone mRNA in their ova, while this mRNA is absent in the ova of cidaroids, asteroids, and holothurioids. Tennent (1914, 1922) and Schroeder (1981) emphasized other aspects of the early development of cidaroids that differ from euechinoids (Table II). Together with the studies on cidaroid development by Mortensen, the present description of the formation of juvenile structures and metamorphosis of *E. thouarsi* shows that cidaroids differ markedly from euechinoids in later development as well (Table II).

The five pairs of lobes found on cidaroid larvae are similar in construction, occur in the same positions, and therefore are probably homologous with the epidermal lobes on euechinoid larvae. The pairs of dorso-posterior and ventro-posterior lobes and the pair of posterior lobes are located in the same positions as the vibratile lobes found on arbaciid, echinometrid, toxopneustid, and some clypeasteroid larvae. The pair of antero-dorsal lobes are in a position similar to the anterodorsal lobes on larvae of arbaciids and some spatangoid echinoids. In these last named euechinoid groups, the anterodorsal and posterior lobes contain skeletal rods that branch from existing spicules. With spicules present, these lobes are long and narrow extensions of the ciliated band and are called extra larval arms (*e.g.*, anterodorsal and posterolateral arms of arbaciids and spatangoids). These comparisons between cidaroid and euechinoid lobes and arms indicate that all pluteus arms may have originated from lobes.

The antero-ventral lobes of cidaroid larvae are poorly developed in other echinoid larvae and usually are evident only as small protrusions or bends between the postoral arms. Similarly, the lateral flaps on the preoral hood region of cidaroids are present in some euechinoid larvae in a reduced form of small bends in the ciliated band (*e.g.*, Strathmann, 1971).

The absence of an amniotic invagination and sac is one of the most striking features of development of *Eucidaris thouarsi*. In all euechinoids with feeding larvae, the oral and adoral structures of the juvenile develop in an amniotic sac and do not occur externally on the left surface until or just prior to metamorphosis (Harvey, 1956; MacBride, 1903; von Ubisch, 1913). The amniotic sac first forms as an inpocketing on the left larval surface during the six-armed stage. This epidermal invagination enlarges, contacts the growing left hydrocoel, and together these tissue layers form the oral surface of the juvenile inside the amniotic cavity (Hyman, 1955). The only euechinoid species with feeding larvae that do not form an amniotic sac by invagination are several (and possibly all) temnopleurids, *Genocidaris maculata*, *Temnopleurus hardwickii*, and *T. toreumaticus* (von Ubisch, 1959; Fukushi, 1959, 1960). During the four-armed larval stage of these species, a mass of cells buds off by invagination from the ectoderm of the left surface

Table II

Differences in the development of cidaroid and euechinoid larvae

Cidaroids	Euechinoids
Hyaline layer is very thin. Consequently, blastomeres do not adhere closely during early cleavages. E.tr., P.b. (Mortensen, 1938; Schroeder, 1981).	Hyaline layer is relatively thick. Blastomeres are tightly adherent.
α-subtype histone mRNA absent from eggs, E.tr. (Raff *et al.*, 1984).	Maternal, α-subtype histone mRNA present in egg nucleus.
Two to three micromeres of variable size and equivalent numbers of macromeres, E.tr. (Schroeder, 1981).	Four equivalent sized micromeres and four equivalent sized macromeres.
No apical tuft forms after hatching, E.tr. (Schroeder, 1981).	Apical tuft present.
Slow development to pluteus, C.c. E.th., E.tr. (Prouho, 1887; this study; Tennent, 1914; Schroeder, 1981).	Development is faster in Lytechinus and Tripneustes (Tennent, 1914, 1922) and in other euechinoids (Amy, 1983; Emlet *et al.*, 1987).
Mesenchyme first forms at the tip of the archenteron mid way through gastrulation, E.tr., E.th. (Tennent, 1914; Schroeder, 1981; this study).	Primary mesenchyme appears on the vegetal plate prior to gastrulation, secondary mesenchyme appears in a manner similar to mesenchyme of cidaroids.
Location of initiation of skeleton is more medial, E.m., E.th., E.tr. (Mortensen, 1937; this study; Tennent, 1914).	Skeleton is initiated at the posterior end of the gastrula on the vegetal plate.
Shape of gastrula/prism stages is elongate, the organization of the ciliated band resembles the asteroid larva of *Asterias*, E.tr. (Tennent, 1914).	Gastrula is only slightly elongate. Prism is not asteroid like.
Formation of 5 pairs of epidermal lobes, E.m., E.th., P.b. (Mortensen, 1937, 1938; this study).	Many species possess epidermal lobes called vibratile lobes (2 pair) as well as other epidermal lobes, though never in the same high degree of development (see text).
Formation of lateral flaps on the preoral hood, C.c., E.th., P.b. (Prouho, 1887; this study; Mortensen, 1938).	Lateral flaps reduced or absent.
Podia grow directly out of the left surface of the larval body, E.th. (this study).	All feeding larvae studied have an amniotic sac inside of which podia and adult spines form.
Epineural space forms between an external epithelium and the epidermis of the oral disk. The juvenile morphology is similar to euechinoids, but the process may differ, E.th. (this study).	Epineural folds form between primary podia and fuse to create an epineural cavity. Described for several species Echinidae and one spatangoid, see text for references.
Metamorphosis is simple, no reorganization of the larval spicules or drastic contraction of the larval epidermis onto the right (aboral) surface of the larva (juvenile), E.th. (this study).	Metamorphosis involves eversion of the oral surface (podia and adult spines) through the vestibular opening and contraction of larval epidermis and larval skeletal elements to the right (aboral) surface of the larva (juvenile).
Much of larval epidermis retained by juvenile, E.th. (this study).	Larval epidermis is histolyzed. Juvenile epidermis from vestibular walls.
Numerous juvenile spines, E.th., P.B. (this study; Mortensen, 1938).	Few or no juvenile spines.
Absence of adult spines at metamorphosis, E.th., P.b. (this study; Mortensen, 1938).	Well-developed adult spines form around the juvenile oral surface within the vestibular cavity.

Traits of cidaroids are compiled from various species, identified by the following abbreviations: C.c., *Cidaris cidaris;* E.m., *Eucidaris metularia;* E.th., *Eucidaris thouarsi;* E.tr., *E. tribuloides;* P.b., *Prionocidaris baculosa.* Traits of euechinoids are generalizations from a large body of literature, including regular and irregular forms.

of the larva and migrates to a position where it contacts the left hydrocoel (von Ubisch, 1959; Fukushi, 1960). Where this mass of cells and the left hydrocoel contact, an amniotic cavity forms and podia and definitive spines of the juvenile develop, as in other euechinoids, enclosed in a cavity (Fukushi, 1960).

Within the amniotic sac of euechinoids (*Psammechinus miliaris,* Theel, 1902; *Echinus esculentus,* MacBride, 1903; *Paracentrotus lividus,* von Ubisch, 1913; *Echinocardium cordatum,* MacBride, 1918), folds of epidermal tissue form between the podia and grow toward the center of the juvenile oral surface. These "epineural folds" are described and figured as "free edges" raised above the floor of the amniotic sac or oral disc (Theel, 1902 plate I, sections 49, 50, & 51; MacBride, 1903 p. 304 and Figs. 40 & 41b; von Ubisch, 1913 Figs. 7 & 8). These folds join along their lateral edges and along their edges central to the oral disc. They enclose an epineural cavity external to the original floor of the amniotic sac. These epineural folds thus enclose the ambulacra and superficial cavity under a double layered epithelium (MacBride, 1903; Hyman, 1955). Sections through the developing oral disc region of euechinoids indicate that the double layered epithelium fits loosely over the epineural cavity, and there is often a large gap between the two epithelial layers (MacBride, 1903 Figs. 44 & 46; von Ubisch, 1913 Figs. 9 & 10). Mesenchyme cells that will form adult test plates migrate into the region between the double layers of epithelium. The epineural cavity and underlying oral disc tissue become restricted to areas along the ring canal and the radial canals of the hydrocoel (von Ubisch, 1913).

Enclosure of the ambulacra also occurs in *Eucidaris*

thouarsi, but differs from euechinoids in the pattern by which the epithelial cells cover the oral disc region. The different patterns may represent different cellular mechanisms of tissue formation. In *Eucidaris thouarsi,* because the most medial region of the covering epithelium is very thin and in contact with the epidermis of oral disc, the epineural cavity appears to be formed by separation of the epithelium from the underlying epidermis. The more peripheral parts of the covering epithelium is double layered, but the space between these layers is never large and does not persist. The double layered epithelium does not appear to be loosely fitting. In *E. thouarsi* no external evidence of epineural folds was seen, nor did the external surface ever appear loosely fitting in the developing oral region. I know of no similar SEM observations on euechinoids, but von Ubisch (1913) presented schematic drawings of ambulacral enclosure which suggested that the epineural folds should be visible within the vestibule.

Observations of ambulacral enclosure in *Eucidaris thouarsi* are consistent with two different, potential mechanisms. An epithelium may migrate from between podial buds toward the center of the oral disc, its leading edge in contact with and "crawling" across the underlying epidermis. In this way, the leading edge drags along an epithelium that is doubled because one part of the epithelium remains anchored at the periphery of the oral disc. After the leading edge passes a location, an epineural space forms by delamination of the epithelium and the underlying epidermis. Alternatively, the epithelium with underlying cavity could form by the spreading of separate cavitation sites that begin peripherally and move toward the center of the oral disc. In the older regions of separation of the two tissues, the superficial epithelium has divided again to form two layers. Detailed ultrastructural work on *both* groups of echinoids is required to distinguish between these two possible mechanisms of ambulacral enclosure and determine how the mechanisms of ambulacral enclosure may differ between cidaroids and euechinoids.

My observations of *Eucidaris thouarsi* on the formation of the radial canals and of secondary podial buds, on the formation of the nerve ring and the radial nerves from oral disc tissue, and of interradial ingression of the left posterior coelom to form dental sacs were similar to those reported for regular euechinoids (von Ubisch, 1913).

Few embryonal or juvenile spines form on the epidermis of euechinoid larvae (*e.g.,* Fukushi, 1960; Onoda, 1931; Emlet, unpubl. obs.). Usually these spines have a characteristic structure with three or four points at their tips, and grow at the posterior end, on the dorsal surface (near the dorsal arch), or on the right side of the larval body (near posterodorsal and postoral spicules). Larvae of certain euechinoid groups (*e.g.,* arbaciids, spatangoids, and clypeasteroids) lack juvenile spines on the larval surface altogether. In contrast, larvae of *Eucidaris thouarsi* possessed approximately 20 juvenile spines on the larval exterior prior to metamorphosis. Mortensen's (1938) figures of late stage larvae and newly metamorphosed juveniles of *Prionocidaris baculosa* indicate similar large numbers of juvenile spines for this species as well.

Advanced larvae of all euechinoids possess definitive or adult spines growing around the adoral juvenile surface within the amniotic cavity. (In *Arbacia* spp. these spines are spatulate rather than columnar.) Definitive spines were absent from newly metamorphosed juveniles of *Eucidaris thouarsi* and *Prionocidaris baculosa* (Mortensen, 1938). The absence of definitive spines in cidaroids at metamorphosis suggests heterochrony in spine formation and may be developmentally correlated with the lack of an amniotic cavity (see below).

In euechinoid metamorphosis, the eversion of the rudiment and contraction of the larval epidermis brings larval spicules and tissues to the aboral surface of the juvenile (right side of larva) and exposes the oral surface and definitive spines. Most of the larval epidermis on the aboral surface is resorbed and histolyzed, and the tissue of the vestibular walls becomes the juvenile epidermis (Bury, 1895; MacBride, 1903; Cameron and Hinegardner, 1978; Chia and Burke, 1978). The very different metamorphosis of *Eucidaris thouarsi* does not involve as drastic a rearrangement of larval tissues and skeletal elements. In cidaroids, external juvenile structures develop on the larval body. No major rearrangements of larval skeletal elements are required to establish juvenile morphology. Only slight movements accompany considerable growth of the bases of larval spicules to set up aboral (apical) plate arrangements in the juvenile. With no amniotic sac and sac lining in cidaroids, the larval epidermal lobes and arms are resorbed in place into the juvenile surface, and the rest of the larval epidermis remains intact to become juvenile epidermis.

Implications for development

This study shows that development and metamorphosis of feeding larvae varies among echinoids and can no longer be generalized from the early descriptions of euechinoid (Echinidae) development by Bury (1895), MacBride (1903), and von Ubisch (1913). Mortensen (1921, p. 230) warned against considering the larvae of the Echinidae to be generally representative of the echinoids, but his advice has largely gone unheeded. Raff (1987) argued that development of feeding larvae of echinoids is highly constrained relative to development of nonfeeding larvae. To the extent that direct developing larvae show greater variation in the timing of initiation

of adult structures, this may be true. However, this study along with the above mentioned studies on temnopleuroids indicates that considerable variation in timing and mode of formation of the amniotic sac also occurs in feeding echinoid larvae. This flexibility within the Class is distributed along taxonomic lines.

The differences between cidaroid and euechinoid development indicate that different developmental pathways can be taken to reach the same objective: a juvenile sea urchin. The lack of an amniotic sac in cidaroids has no lasting effect on the post metamorphic juvenile, but has a profound affect on the complexity of metamorphosis and on the fate of some larval and early juvenile structures. With an amniotic sac metamorphosis is relatively complex; without it, metamorphosis is simpler. The amniotic sac of euechinoids is important in metamorphosis because it is the site of formation of definitive structures, such as adult spines and epidermis. In cidaroids the absence of a vestibule is correlated with a different fate for the larval epidermis and with the absence of adult spines. The absence of an amniotic sac may also account for some of the differences reported here on the enclosure of the ambulacra. Inside an amniotic sac, podial buds are forced to grow toward the center of the oral disc. Euechinoid epineural lobes may be exaggerated by being restricted to areas between podial buds which are crowded together by the roof of the amniotic sac.

The differences in spine formation between euechinoids and cidaroids indicate that some compensation by different developmental processes may be occurring. The presence in cidaroids of a large number of juvenile spines may compensate for the lack of definitive spines at metamorphosis. Conversely, presence in euechinoids of definitive spines within the amniotic sac may reduce or eliminate the need for juvenile spines at metamorphosis. The delayed formation of adult spines in cidaroids is another example of differences in timing of developmental events. In euechinoids, an amniotic sac with its lining of adult epidermis may permit the precocious formation of definitive spines around the oral surface of the juvenile that might otherwise interfere with normal functions of swimming and feeding by the pluteus. Strathmann (in press) discussed numerous other developmental events of echinoderms that may not be independent.

Implications for phylogenetic studies

The amniotic invagination must now be considered both present and absent in the class Echinoidea. The absence of an amniotic invagination and the pattern of ambulacral enclosure in cidaroids raises further questions about the utility of certain ontogenetic characters in establishing relationships between Classes and about the origin of the amniotic sac of euechinoids. The characters described for cidaroid larvae could readily be interpreted as primitive among echinoids, but polarizing them as such requires comparison with a sister group to the echinoids. Regardless of the choice of sister group, convergence of developmental characters among the classes occurs frequently (see Strathmann, in press).

Though relationships between classes of echinoderms are still uncertain, several recent phylogenies of echinoderms have echinoids and holothuroids as sister groups (Smith, 1984b; Raff *et al.*, in press) or echinoids and ophiuroids as sister groups (Smiley, in press; but also see Strathmann, in press). Both of these possible choices for sister groups to the echinoids develop the adult mouth at the site of the larval mouth. In contrast, echinoids and asteroids develop the adult mouth on the left side of the larval body, independently of the larval mouth. The present study suggests cidaroid echinoids and asteroids are even more similar in development, because both lack epidermal invaginations at the site of formation of the adult mouth. The great differences in adult morphology and other differences in larval morphology make it unlikely that these striking similarities between asteroid and cidaroid larvae are due to a shared ancestor, but rather due to morphological convergence.

The description of ambulacral enclosure given here for *Eucidaris thouarsi* is quite similar to that described for the ophiuroid *Ophiopholis aculeata* (Olsen, 1942). In describing the epithelial tissue that covered the oral epidermis, Olsen (p. 81) stated that the inner layer of the double layered tissue was at first in contact with, but later separated from, the underlying epidermis. The cavity that formed was the epineural canal. Earlier studies on ophiuroid development (MacBride, 1907; Narasimhamurti, 1933) report epineural folds enclosing the ambulacra but are not clear on the details of the relationship of epithelial and underlying epidermal tissue layers. Since epineural folds were first observed in ophiuroids, they have been considered homologues of the epineural folds in euechinoids (MacBride, 1907; Hyman, 1955). If the description of ambulacral enclosure for *Eucidaris thouarsi* generalizes to all cidaroids and the description for *Ophiopholis aculeata* generalizes to all ophiuroids, the similarity may reflect common origin. This similarity may also be convergent and due to the open surfaces on which these processes are occurring in ophiuroids and cidaroids.

The term *vestibule* refers to an epidermal invagination on the larva at the site of the adult mouth, around which the water vascular system develops (Ubaghs, 1967). The implicit function of the vestibule is to bring epidermal tissue into contact with hydrocoelic tissue to initiate formation of adult oral structures. This term is used to describe the oral cavity of holothuroid and ophiuroid larvae because the left hydrocoel of these larvae grows around the esophagus just beneath the epidermis of the

oral cavity (Smiley, 1986; Olsen, 1942). The highly modified, non-feeding larvae of crinoids possess an invagination identified as a vestibule, but its relationship to larval structures is uncertain. An indication that this vestibule is a vestige of the larval mouth is implied by Lacalli and West's (1986) study of transitional patterns of ciliation in the crinoid larva of *Florometra serratissima*. The same term is used to describe the amniotic invagination and sac on the left side of the larval body of euechinoids. Because of the involvement of these epidermal invaginations in formation of the adult mouth, Hyman (1955) suggested they were homologous across the phylum. Hyman's view of homology of vestibules ignores the fact that the amniotic sac of euechinoids is not associated with the larval mouth but is located on the left side of the larval body. The vestibule of other classes and the amniotic sac of euechinoids also differ morphologically because this later invagination is completely sealed off from the exterior during a portion of development. Based on positional and morphological criteria, the amniotic sac of euechinoids should not be considered homologous with the vestibule of other echinoderms. This conclusion is paradoxical, because the adult mouths of extant echinoderms are considered homologous.

There is another line of evidence that suggests the euechinoid amniotic sac is not homologous with the vestibule of other classes. Larvae from the four non-echinoid echinoderm classes and probably all cidaroids retain much of the larval epidermis to form adult epidermis (Chia and Burke, 1978). In contrast, euechinoids histolyze much of the larval epidermis and replace it with adult epidermis that originates from the amniotic sac. The similar fate of the larval epidermis in non-echinoid classes and cidaroids implies that the condition found in euechinoids is derived and is permitted by the amniotic sac. This line of evidence suggests that the euechinoid amniotic invagination is a derived character within the echinoids and that its absence in cidaroids is primitive among the Echinoidea.

If the absence of an amniotic sac in cidaroids is primitive among echinoids, the amniotic sac of euechinoids is either an independently evolved structure or an example of incomplete evolutionary reversal. Similarities between the amniotic sac of euechinoids and the vestibules of other echinoderms suggest that an evolutionary reversal is more likely. A functional role for the amniotic sac of euechinoids as a site for precocious formation of adult epidermis, adult spines, and juvenile rudiment is compatible with this origin.

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